target mRNA in the mitochondrion occurs, thereby inhibiting expression of the target mitochondrial gene in the cell.

[0014] Additional aspects of this invention include a method of treating dystrophia myotonica (DM) in a subject, comprising administering to the subject an effective amount of the RNA endonuclease of this invention, wherein the RNA binding domain of the RNA endonuclease is modified to bind mRNA encoding (CUG)n repeats in the 3' UTR of DMPK to treat DM1 and/or mRNA encoding (CCUG)n repeats in intron 1 of ZNF9 to treat DM2 and wherein the RNA endonuclease comprises a mitochondrial targeting signal sequence, thereby treating DM in the subject.

[0015] The present invention also provides a method of detecting an RNA virus in a sample, comprising: a) contacting the sample with the RNA endonuclease of this invention under conditions whereby cleavage of RNA occurs if RNA of the RNA virus is present in the sample and wherein the RNA binding domain of the RNA endonuclease is modified to bind a target RNA of the RNA virus; and b) detecting a cleavage product of the target RNA, thereby detecting the RNA virus in the sample.

[0016] A method is also provided herein of diagnosing a viral infection in a subject, comprising: a) contacting the sample from the subject with the RNA endonuclease of this invention under conditions whereby cleavage of RNA occurs if viral RNA is present in the sample and wherein the RNA binding domain of the RNA endonuclease is modified to bind viral RNA; and b) detecting a cleavage product of the target RNA, thereby detecting viral RNA in the sample and thereby diagnosing a viral infection in the subject.

[0017] Furthermore, the present invention provides a method of identifying a strain of an RNA virus in a sample, comprising: a) contacting the sample with the RNA endonuclease of this invention under conditions whereby cleavage of RNA occurs if the RNA of the strain of the RNA virus is present in the sample and wherein the RNA binding domain of the RNA endonuclease is modified to bind a target RNA specific to the strain of the RNA virus; and b) detecting a cleavage product of the target RNA, thereby identifying the strain of the RNA virus in the sample.

[0018] The foregoing and other aspects of the present invention will now be described in more detail with respect to other embodiments described herein. It should be appreciated that the invention can be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

DESCRIPTION OF THE FIGURES

[0019] FIG. **1**. Design and activity validation of artificial sequence specific RNA endonucleases (ASREs). (Panel A) Structures of the PUF domain of pumiliol with NRE-19 RNA (1M8W) and the PIN domain of Smg6 (2HWW). The PUF domain contains eight repeats, each recognizing a single RNA base. Three amino acids from each repeat interact with the Watson-Crick edge of an RNA base and determine the binding specificity of the repeat (left panel). The PIN domain has an RNase H like active site with three Asp residues and co-ordinates one divalent metal cation (right panel). (Panel B) ASRE with N-terminal PUF, C-terminal PIN and a heptapeptide linker was incubated with a cognate RNA substrate for 30 min. Site-specific cleavage of RNA was obtained with the ASRE containing a wild type PIN domain (lane 2) but not with ASRE containing a mutated PIN domain (lane 3). (Panel C) Inverted ASRE (PIN-PUF fusion protein from N to C terminus) caused complete, non-specific cleavage of an RNA substrate. The ASRE in PUF-PIN orientation was included as control (lane 1).

[0020] FIG. **2**. Biochemical characterization of ASREs. (Panel A) The linker length affects the activity of ASRE. ASRE with a tripeptide linker showed limited activity whereas efficient cleavage activity was achieved with medium (heptapeptide) and long linkers (dodecapeptide). At longer reaction time, non-specific digestion products (indicated by asterisks in lane 5) were observed with ASRE containing a dodecapeptide linker. (Panel B) Digestion time course of 7u6g RNA by ASRE(6-2/7-2). Digestion was followed in the standard reaction condition substituted with 3 mM Mn^{2+}. (Panel C) RNA substrates containing either an NRE site (UGUAUAUA) or 7u6g site (UugAUAUA) were incubated with ASRE(Wt) or ASRE(6-2/7-2). Lanes 1 and 4 are controls without enzyme. (Panel D) ASRE shows divalent metal ion selectivity, with Mn^{2+} yielding highest activity and Mg^{2+} and Co^{2+} giving lower activity. The concentrations of divalent metal ions were 3 mM in all lanes. (Panel E) Semilog plots were used to determine the pseudo first-order reaction rates of ASRE catalyzed RNA cleavage in the presence of 2.5 mM (♦), 5 mM (■) and 7.5 mM (▲) Mn^{2+}. Non-specific digestion occurred with Mn^{2+} concentration greater than 7.5 mM (data not shown).

[0021] FIG. **3**. Kinetic parameters and cleavage sites of ASREs. (Panel A) Plot of initial reaction rates vs. substrate concentration for ASRE(wt). The enzyme was incubated with various concentration of cognate substrate for 5 min and the initial reaction rates were measured as the amount of RNA cleaved per minute. Kinetics constants were determined by fitting the curve to a Michaelis-Menton kinetic model. (Panel B) Plot of initial reaction rates vs. substrate concentration for ASRE(6-2/7-2). Reaction conditions and data analysis were the same as panel A. (Panel C) The cleavage site was mapped by 5' or 3' DSS-RACE from gel purified RNA products. The positions of two cleavage sites are indicated with arrows and the relative frequencies are plotted. The 8-nt binding sequence of PUF is shown in bold. (Panel D) The "curve back" model of ASRE best explains the cleavage positions mapped in panel C.

[0022] FIG. **4**. Using ASRE to silence gene expression in bacterial cells. (Panel A) The activity of β-galactosidase in *E coli* strains transformed with expression plasmids of ASRE(lacZ) and control ASREs. Expression of ASRE and endogenous lacZ gene was induced with IPTG. For each ASRE strain, lacZ expression for five independent clones (N=5) over three experiments (in triplicates) was measured to circumvent clonal variation. The percent of β-galactosidase activity was normalized to clones transformed with the empty vector (N=2) and induced with the same condition. The β-galactosidase activity was also measured for uninduced clones containing empty vector as the baseline activity. Control ASREs include the non-specific ASRE(87621) that targets a different sequence and the mutated ASRE (LacZ) containing a D1353A mutation in the PIN domain active site. (Panel B) The levels of lacZ mRNA were measured by real time RT-PCR. The cell samples were the same as in panel A. LacZ mRNA levels were normalized to ftsZ mRNA and the relative RNA abundances compared to